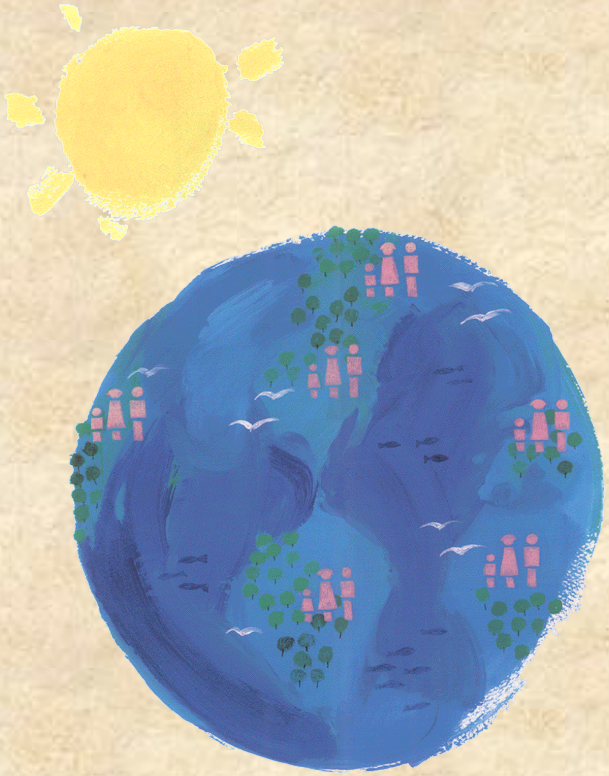
NICEATM

National Toxicology Program Interagency Center for the Evaluation Of Alternative Toxicological Methods

ICCVAM

Interagency Coordinating Committee on the Validation of Alternative Methods



The Bovine Corneal Opacity and Permeability (BCOP) Test Method

BRD Summary

Expert Panel Meeting
January 11-12, 2005
Bethesda, Maryland

National Toxicology Program

NIEHS

Current U.S. Regulatory Status of BCOP

- ICCVAM agencies were surveyed
- BCOP data was submitted to EPA and FDA for consideration

Primary BCOP Data Sources

Study	Accuracy (S/NS)			Intralab (S/NS)	Interlab (S/NS)	
	GHS	EPA	EU	CVs	CVs	GHS class.
Gautheron et al. (1994)	7/6	6/6	8/43	-	52	7/6
Balls et al. (1995)	22/35	20/35	21/38	-	59	22/35
Swanson et al. (1995)	6/3	6/3	5/4	20[a]	-	-
Gettings et al. (1996)	8/17	10/15	6/19	25[b]	-	-
Casterton et al. (1996)	26/30	26/29	24/36	-	-	-
Southee (1998)	6/8	6/8	5/10	16[c]	16	6/8
Swanson and Harbell (2000)	1/8	4/5	1/8	-	-	-
Bailey et al. (2004)	3/13	3/13	3/13	-	-	-
Dr. Joseph Sina's submission	-	-	-	29[d]	-	-

S = severe or corrosive irritants; NS = nonsevere irritants or nonirritants; class. = classification.

[a] Intralaboratory repeatability was evaluated (n=5 corneas). Data received after publication of draft BRD. [b] Intralaboratory reproducibility was evaluated (n=3 replicate experiments). [c]Intralaboratory repeatability and reproducibility were evaluated. [d] Intralaboratory repeatability was evaluated (n=4 corneas).

*_In vivo_ data not available for this study.

Other BCOP Reports Considered

- 31 other reports were identified that could not be used for an evaluation of accuracy or reliability due to the lack of:
  - appropriate comparative *in vivo* rabbit test data (i.e., raw scores for individual animals)
  - quantitative *in vitro* data
  - adequate information on test substances
  - *in vivo* data obtained from currently accepted regulatory test guidelines
- These reports discussed in Section 9
- To the extent possible, data requested from authors of studies considered most useful

Database

- 166 different substances or formulations evaluated
- 15 Chemical classes tested*
  - Most frequent classes: formulations, alcohols, heterocyclic compounds, acids and ketones
- 20 Product classes tested*
  - Most frequent classes: solvents, chemical/synthetic intermediates, drugs/pharmaceuticals, petroleum products, cleaners, personal care cleansers, and hair shampoos

*Classes with at least 3 entries

Test Method Protocol Variations

- BCOP test method protocols were similar to each other, but not identical
- Examples of test method components that differed among protocols used to generate data include:
  - Storage conditions of bovine eyes during transport (e.g., use of antibiotics, ambient temperature vs. over ice)
  - Use of MEM with or without phenol red for incubations
  - Instrument used to measure opacity (opacitometer vs. spectrophotometer)
  - Use of positive controls
  - Use of different negative controls (e.g., saline vs. sterile deionized water)
  - Application of solid test substances (20% suspension vs. neat substance)
  - Analysis of resulting data
  - Addition of other endpoints, such as histology

BCOP Data Analysis Methods

- *In Vitro* Irritancy Score (IVIS)*
  - IVIS = mean opacity value + (15 x mean OD_{490} value)
  - IVIS > 55.1 = severe irritant
- Endpoint with highest score**
  - Permeability > 0.600 = severe irritant
  - Opacity > 1.300 = severe irritant
- Permeability value only
  - Some studies analyzed permeability data only for substances that produce significant permeability without appreciable opacity
    - anionic and nonionic surfactants
    - some surfactant-based personal care formulations
- Comparison to benchmark substances

*Used in protocols that measure opacity with an opacitometer.
**Used in protocols that measure opacity with a UV/VIS spectrophotometer.

Distribution of Tests Among Analysis Methods*

Method	Number of Testing Laboratories			
	1	3	5	11/12
In Vitro Irritancy Score	28	16	51	52
Opacity or Permeability	60			
Permeability only	25			
Comparison to benchmark	9			

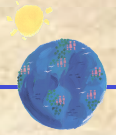
*Includes only tests for which *in vivo* data were available.

BCOP Accuracy Analysis

- Ability of analysis methods to correctly identify ocular corrosives and severe irritants determined for
  - GHS classification system (Category 1)
  - EPA classification system (Category I)
  - EU classification system (R41)

- Accuracy statistics calculated for:
  - each BCOP study with *in vitro* and *in vivo* data
    - by test substance
    - by test
  - pooled data from studies with similar protocols

- False negative and false positive rates calculated by chemical class and available physicochemical properties (liquid/solid)



Overall BCOP Accuracy Results*

Statistic	GHS (n=120)*		EPA (n=117)*		EU (n=157)**	
	%	n	%	n	%	n
Accuracy	79	95/120	80	93/117	77	121/157
Sensitivity	76	32/42	73	33/45	77.5	31/40
Specificity	81	63/78	83	60/72	77	90/117
False Positive Rate	19	15/78	17	12/72	23	27/117
False Negative Rate	24	10/42	27	12/45	22.5	9/40

*BCOP data from the the following studies were pooled for this analysis: Gautheron et al. (1994), Balls et al. (1995), Swanson et al. (1995), Gettings et al. (1996), Swanson & Harbell (2000), Bailey et al. (2004).

**Additional 37 chemicals available for EU analysis only (individual animal data not available for GHS or EU classification)

BCOP False Negative & False Positive Rates by Chemical/Physical Class (GHS)

Category	n	False Negative Rate		False Positive Rate	
		%	No.	%	No.
OVERALL	120	24	10/42	19	15/78
Formulations with surfactants	34	14	2/14	5	1/20
Alcohol	10	100	1/1	44	4/9
Formulation, ethanol containing	8	0	0/1	29	2/7
Surfactant, cationic	7	0	0/6	0	0/1
Acetate	6	-	-	0	0/6
Formulation, petrochemical	6	33	1/3	0	0/3
Acid	5	0	0/3	50	1/2
Heterocyclic compound	5	25	1/4	0	0/1
Surfactant, nonionic	4	-	-	50	2/4
Aromatic hydrocarbon	3	-	-	0	0/3
Inorganic chemical	3	50	1/2	0	0/1
Ketone	3	-	-	67	2/3
Alkali	2	0	0/1	100	1/1
Amine; Cyclic Hydrocarbon; Oil	2	-	-	0	0/2

BCOP False Negative & False Positive Rates by Chemical/Physical Class (con't)

Category	n	False Negative Rate		False Positive Rate	
		%	No.	%	No.
Petrochemical, cutting fluid	2	-	-	0	0/2
Polycyclic aromatic hydrocarbon	2	0	0/2	-	-
Surfactant, anionic	2	100	1/1	0	0/1
Acyl halide; Amide	1	-	-	0	0/1
Aldehyde; Organophosphate	1	-	-	100	1/1
Amidine	1	0	0/1	-	-
Chlorinated hydrocarbon; Lactone	1	-	-	0	0/1
Diol		100	1/1	-	-
Organometallic	1	-	-	0	0/1
Organophosphate	1	-	-	100	1/1
Quaternary ammonium surfactant	1	100	1/1	-	-
Terpene; Wax	1	-	-	0	0/1
Thiophthalimide	1	100	1/1	-	1
Liquid	94	18	5/28	21	14/66
Solid	19	33	4/12	29	2/7

Limitations of BCOP Accuracy Analysis

- For a majority of the chemical classes (63%; 20/32), only a small number (≤2) of substances were tested
- Only 6 chemical classes for which ≥5 substances were evaluated in BCOP
- Limited information about physicochemical (e.g., solid, liquid) properties for some test substances
- A few substances were tested in multiple *in vivo* studies that produced different *in vivo* classifications; the most severe classification was used for the analysis
- Individual rabbit eye data not available for Gautheron 1994 interlaboratory study at time of draft BRD publication; data just received, which will provide additional information for GHS and EPA accuracy analyses

BCOP Reliability Analysis

- Intralaboratory Repeatability and Reproducibility
  - Quantitative analysis: Coefficient of variation
- Interlaboratory Reproducibility
  - Qualitative analysis: Extent of agreement between testing laboratories when identifying corrosives and severe irritants
  - Quantitative analysis: Coefficient of variation

BCOP Intralaboratory Repeatability - %CV Values for *In Vitro* Irritancy Score

	%CV	Dr. Sina Submission	Swanson et al. 1995*	Southee 1998 (Lab 1)	Southee 1998 (Lab 2)	Southee 1998 (Lab 3)
All Data	Mean	71	-44	48.3	39.2	30.5
	Median	35	7.9	14.2	11.8	12.4
	Range	1.1 - 479	-782 - 32.5	0.1 - >500	2.1 - >500	4.3 - >500
Substances Predicted as Severe	Mean	8.2	Not calculated	8.44	8.4	11.1
	Median	8.1	Not calculated	7.05	8.1	9.3
	Range	1.1 - 13	Not calculated	0.1 - 22	2.1 - 21.7	5.1 - 30.3

*Replicate cornea data received December 23, 2004

BCOP Intralaboratory Reproducibility

- Gettings et al. 1996: 25 substances, 3 trials, 1 lab
  - Mean and Median %CV for permeability value was 33.4 and 29
  - Substances spanned a range of irritancy
  - Surfactant-based personal care cleaning formulations

- Southee 1998: 16 substances, ≥ 2 trials, 3 labs
  - Mean %CVs for IVIS ranged from 12.6 to 14.8 for the 3 labs
  - Median %CVs for IVIS ranged from 6.7 to 12.4 for the 3 labs
  - Substances spanned a range of irritancy

BCOP Interlaboratory Reproducibility - Classification Agreement Among Laboratories

% Interlab Agreement	Balls et al. 1995 (5 labs): GHS		Southee 1998 (3 labs): GHS		Gautheron et al. 1994 (11 or 12 labs)	
	%	N	%	N	%	N
100% (all)	68	41/60	94	15/16	71%	36/51
≥80% (all)	85	51/60	94	15/16	91	46/51
100% (severe *in vivo* and *in vitro*)	82	14/17	100	3/3	60	3/5
≥80% (severe *in vivo* and *in vitro*)	94	16/17	-	-	80	4/5

BCOP Interlaboratory %CV Values for *In Vitro* Irritancy Score

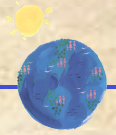
	%CV	Gautheron et al. 1994 (11 or 12 labs)	Balls et al. 1995 (5 labs)	Southee 1998 (3 labs)
All Data	Mean	168 (n=52)	50 (n=50)	32 (n=16)
	Median	46.9 (n=52)	26 (n=50)	23 (n=16)
	Range	16.5 - 1325 (n=52)	7.6 - 712 (n=50)	7.5 - 109 (n=16)
Substances Predicted as Severe	Mean	36 (n=17)	25 (n=32)	11.1 (n=5)
	Median	17 (n=17)	22 (n=32)	8.6 (n=5)
	Range	16.5 - 55.7 (n=17)	7.6 - 89.4 (n=32)	7.5 - 21.6 (n=5)

Limitations of BCOP Reliability Analysis

- No major limitations identified

Draft BCOP BRD Proposals (1)

- A recommended BCOP version identified, which evaluates
  - Opacity and permeability
  - Histology recommended on a case-by-case basis (see Section 2.2.3 of BRD; Appendix A-22)
- A standardized protocol proposed for the recommended version of the BCOP test method
  - Protocol based on the method used by the Institute for *In Vitro* Sciences (IIVS)
  - Only significant difference is that the recommended protocol in the BRD lacks the detailed histology procedures provided in a separate IIVS protocol on histology for the BCOP assay
  - Decision criteria previously described by Gautheron et al. (1994)



Draft BCOP BRD Proposals (2)

- Proposed optimization studies recommended, including:
  - Retrospective analysis of decision criteria used to identify corrosives and severe irritants
  - An evaluation of possible increased interlaboratory variability for specific chemical classes appearing more variable (e.g., alcohols)
  - An evaluation of reduced exposure times for alcohols, and possibly other volatile solvents
  - Determining the utility of histopathology and when it should be included.
- Once optimized, additional validation studies recommended to further assess the accuracy and reliability of BCOP, so that the applicability domain can be better defined and data gaps can be filled in